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FULL LENGTH ARTICLE

Successive solvent extraction and GC–MS analysis for the evaluation of the phytochemical constituents of the filamentous green alga *Spirogyra longata*

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KEYWORDS

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Sterols

Abstract *Spirogyra* species are one of the most common freshwater filamentous green algae reported to have antimicrobial activities. However, their chemical constituents have not been entirely detected. In this work, the chemical constituents of *Spirogyra longata*, successively extracted with petroleum ether, methylene chloride, chloroform, acetone and methanol were determined by GC–MS. The extract percentage varied greatly between different solvents, with the highest one (4.83%) recorded for methanol. A total of 97 compounds were identified in different extracts, out of which there were, 5 alkaloids, 6 ketones, 8 terpenes, 4 phenolics, 20 hydrocarbons, 4 fatty acids, 5 fatty alcohols, 20 esters, 7 sterols and 18 others. Generally, the composition and mass fraction of phytochemical constituents in *S. longata* extracts were affected by the extraction solvent, in which, the ketone hexahydrofarnesylacetone and the phenolic, butylated hydroxytoluene (BHT) were dominant in *S. longata* chloroform extract with peak area% of 17.38 and 50.7. Meanwhile, neophytadiene and phytol were the dominant terpenes in methylene chloride extract with a peak area percentage of 24.32 and 30.43, while, hydrocarbons (C_{10}–C_{29}) and sterols were found to maintain 0.624% and 0.46% of *S. longata* DW and were mainly detected in petroleum ether extract. Alkaloids were only detected in acetone and methanol extracts and comprise 0.399% of *S. longata* DW. Moreover, fatty acids and esters were found to represent about 30% of the total extracts, and were dominated by palmitic acid and its methyl and ethyl esters. In conclusion, *S. longata* was found to contain a variety of valuable compounds, which reflect its pharmaceutical and biofuel potentialities.

Introduction

Filamentous algae are usually considered as 'macrophytes' since they often form floating masses that can be easily harvested. Ecologically, algae are the most widespread of the

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photosynthetic plants, constituting the bulk of carbon assimilation through microscopic cells in marine and freshwater (Ramaraj et al., 2014). *Spirogyra* is one of the most common green filamentous algae (Hasan and Chakrabarti, 2009), and it was reported to possess antimicrobial activities (Junthip et al., 2013; Thumvijit et al., 2013; Yousif et al., 2014). The chemical compounds produced by algae generally include neutral lipids, polar lipids, wax esters, sterols and hydrocarbons, as well as prenyl derivatives such as tocopherols, carotenoids, terpenes, quinines, pyrrole derivatives (e.g. chlorophylls), terpenoids, phlorotannins, steroids, phenolic compound, halogenated ketones, alkenes and cyclic polysulphides (Arisz et al., 2000; Guschina and Harwood, 2006; Rao et al., 2007; Plaza et al., 2009).

Many previous pharmacological studies on algae revealed that the chemical compounds produced by marine algae had different biological activities that included being anti-inflammatory, anticancer, anti-HIV, antimutagenic and scavenging free radicals (e.g. Cornish and Garbary, 2010; Bechelli et al., 2011). The freshwater algae are, also, considered as a promising source of bioactive products (Ozturk et al., 2006). However, the chemical composition of compounds produced by freshwater algae (e.g. *Spirogyra*) is relatively less investigated when compared to that of marine algae (Cannel et al., 1998; Stefanov et al., 1999). Therefore, this work is mainly aimed at evaluating the phytochemical constituents of the filamentous freshwater alga *Spirogyra longata* and to highlight their biological and/or industrial values. The correlation between the extraction solvent and the extracted chemical constituent was also taken into consideration.

Materials and methods

Samples collection and preparation

Biomass samples of the filamentous alga *S. longata* (Vaucher) Kützing were collected from the El Serw agriculture canal (31° 49′02.1″E & 31° 14′49.4″N), Dakahlia, Egypt, on July 2014. The collected biomass was cleaned up from epiphytes and non-living matter, rinsed many times with freshwater and identified according to Prescott (1973). It was then air dried for two days and dried at 40 °C in an oven for 2–3 days till constant dry weight. Dried algal samples were mechanically grinded into a coarse powder to facilitate extraction and placed in clean paper bags as a preparation for solvent extraction processes.

Extraction of chemical constituents

Twenty grams of the dried powdered algal sample was successively extracted by soxhlet apparatus, according to the method adopted by Sadasivam and Manickam (1996) using different organic solvents with analytical reagent (AR) quality. These solvents were petroleum ether (40–60 °C), methylene chloride (39.6 °C), chloroform (61.15 °C), acetone (56 °C), and finally methanol (64.7 °C). To ensure the complete extraction process, exhaustive extraction was applied with each solvent for 10 h. Extracts of different organic solvents were collected separately into dry clean beakers, after that they were recovered from the solvents by evaporation in a rotary evaporator at 60 °C, then were dried in desiccators for 1 h and finally the extracts were weighted and the percentage of each extract was determined as follow:

$$\text{Extract } \% = \text{Weight of extract in grams} / \text{Weight of sample in grams} \times 100.$$

The results were expressed as percentage of DW, where DW = algal dry weight.

The extracts were kept under vacuum desiccators until used for gas chromatography/mass spectrometry (GC–MS) analysis.

Gas chromatography-mass spectrometry (GC–MS)

Different crude extracts of *S. longata* were analyzed by the GC–MS technique. Chemical composition of the crude extracts was determined using an Agilent mass spectrometric detector with a direct capillary interface fused with silica capillary column PAS-5ms (30 mm × 0.25 μm film thickness). Samples were injected under the following conditions: Helium was used as carrier gas at approximately 1 ml/min., pulsed splitless mode. The solvent delay was 3 min and the injection size was 1.0 μl. The mass spectrophotometric detector was operated in electron impact ionization mode with an ionizing energy of 70 eV and scanning from m/z 50–500. The ion source temperature was 230 °C and the quadruple temperature was 150 °C. The electron multiplier voltage (EM voltage) was maintained at 1250 V above auto tune. The instrument was manually tuned using perfluorotributyl amine (PFTBA). The GC temperature program started at 60 °C then elevated to 280 °C at a rate of 8 °C/min, with a 10 min hold at 280 °C. The detector and injector temperatures were set at 280 °C and 250 °C, respectively. Wiley07 and NIST05 (National Institute of Standards and Technology) mass spectral databases were used in the identification of the separated peaks. GC–MS analysis was carried out at The Central Agricultural Pesticide Laboratory (CAPL), Dokki, Giza, Egypt. The percentage dry weight of each chemical group identified in different solvents to *S. longata* dry weight biomass was calculated using the following equation:

$$\text{Concentration of each chemical group(g/100 g DW)} = \left(\sum \text{peak area\% of compounds belonging to this chemical group in an extraction solvent}/100\right) \times \text{extract of this solvent(\%)}.$$

Results and discussion

In the present study, the phytochemical constituents of *S. longata* were successively extracted by five different organic solvents ranging in polarity from 1.0 (petroleum ether) to 5.1 (methanol). The total crude extracts was about 8.7% DW. A comparison of extraction yield in different extraction solvents showed that solvent type had a significant effect on the extraction yield, where, methylene chloride maintained the lowest percentage of extract (0.171%) and methanol maintained the highest (4.83%) (Fig. 1). The chemical constituents in the five different crude extracts of *S. longata* were analyzed by GC–MS. The chromatogram of different crude extracts is shown in Fig. 2. A total of 97 different compounds were identified in the five crude extracts (Tables 1–4). The identified compounds with their IUPAC name, common name, retention time (Rt), molecular formula, % similarity, molecular weight

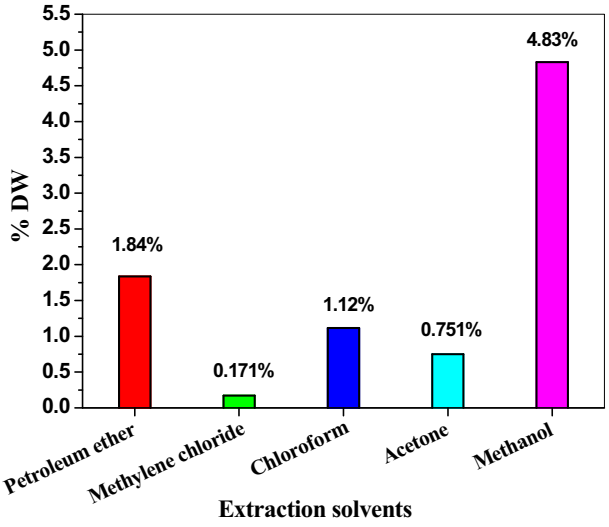

Figure 1 Variation in % crude extracts of the filamentous alga *Spirogyra longata* with different organic solvents.

and % peak area were categorized to ten chemical groups. The percentage of contribution for each identified chemical group to *S. longata* dry biomass is shown in Table 1. The identified chemical groups are ketones, terpenes and phenolics (Table 2), fatty acids (FA), fatty alcohols and esters derived fatty acids and/or dicarboxylic acid (Table 3), hydrocarbons, steroids, alkaloids and others (Table 4). Some of the detected compounds show an unclear fragmentation pattern and low percentage of similarity in matching with compounds in NIST and Willey library, so it was difficult to be identified and hence described as unknown compounds (Table 4). The lowest number of compounds (12 compounds) was identified in the chloroform extract, while the highest one (56 compounds) was identified in the methanol extract (Table 1). The mass spectra of dominant compounds in different extracts of *S. longata* are shown in Fig. 3a and b. For simplicity, the identified compounds belonging to different chemical groups and their relative importance will be addressed separately.

Ketones

In the present investigation ketones were found to represent about 1.94% of algal dry weight (Table 1). This concentration is relatively high representing about 22.6% of the total crude extracts. Out of 6 ketones identified in *S. longata* extracts, the three ketones; 3-penten-2-one, 4-methyl-; 2-pentanone, 4-hydroxy-4-methyl- and hexahydrofarnesylacetone were the dominant with a total peak area% of 20.7, 23.55 and 39.12 (Table 2). Hexahydrofarnesyl acetone was the only ketone detected in all *S. longata* extracts. It represented peak area% of 10.84, 1.76, 17.38, 6.64 and 2.5 of petroleum ether, methylene chloride, chloroform, acetone and methanol extracts (Table 2). Hexahydrofarnesyl acetone is characterized by its fragrant nature with widespread presence in higher plants (Rayne, 2008; Muanda et al., 2010) and has been also identified as lipid component of some algae including the green algae *Mougeotia viridis* (Kamenarska et al., 2000), *Cladophora* (Elenkov et al., 1996; Kamenarska et al., 2004), *Botryococcus braunii* (Abdel-Aal, 2013) and the brown marine alga *Padina pavonia* (Kamenarska et al., 2002).

Terpenes

As shown in Table 2, terpene compounds were detected in all *S. longata* extracts, but with different concentrations that varied

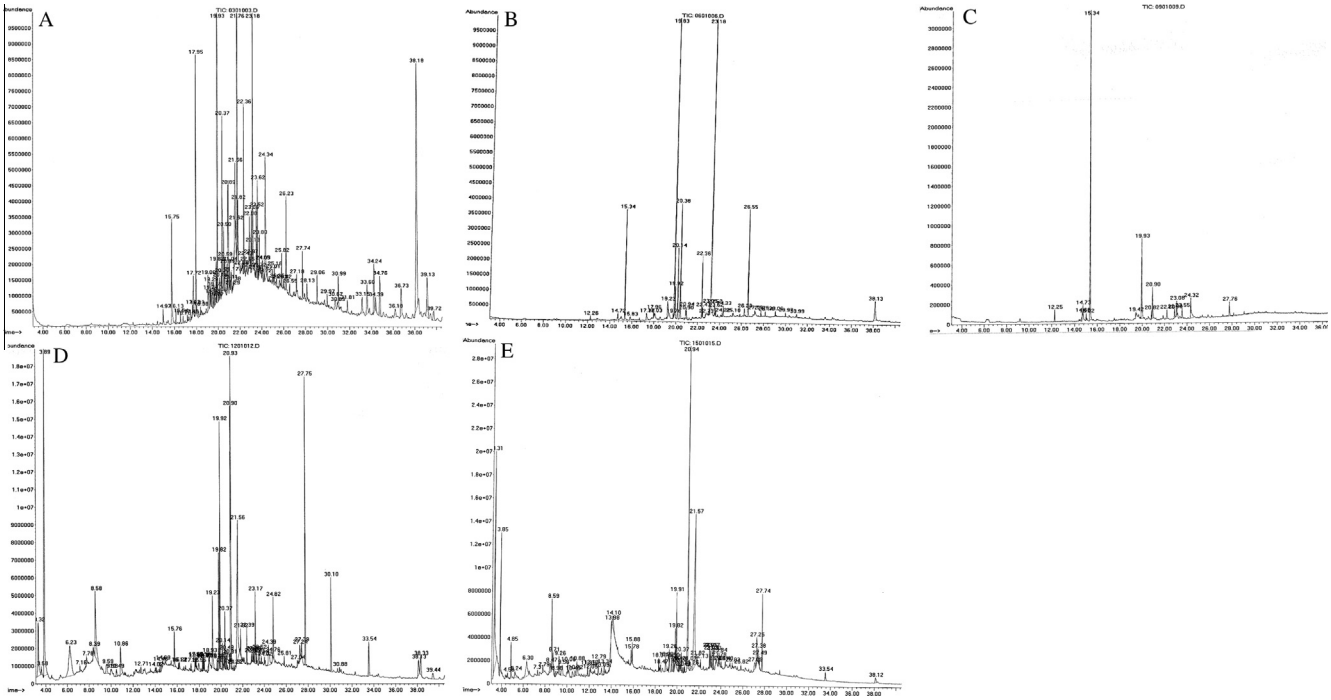

Figure 2 Chromatogram of different crude extracts of petroleum ether (A), methylene chloride (B), chloroform (C), acetone (D) and methanol (E).

Table 1 % Contribution of each chemical group identified in different solvents to *Spirogyra longata* dry weight biomass.

Identified chemical groups	%					
	Extraction solvents					Sum
	Petroleum ether	Methylene chloride	Chloroform	Acetone	Methanol	
Ketones	0.199 **(1)**	0.003 **(1)**	0.195 **(1)**	0.217 **(6)**	1.323 **(5)**	1.937
Terpenes	0.184 **(5)**	0.098 **(4)**	0.024 **(2)**	0.086 **(8)**	0.236 **(5)**	0.629
Phenolic	0.007 **(1)**	0.028 **(3)**	0.619 **(3)**	–	–	0.654
FA	0.1056 **(2)**	0.0011 **(1)**	–	0.0864 **(3)**	1.024 **(4)**	1.217
Fatty alcohols	0.133 **(4)**	0.0199 **(2)**	–	0.025 **(2)**	0.061 **(3)**	0.239
Esters	0.381 **(12)**	0.005 **(5)**	0.199 **(5)**	0.098 (**11**)	0.695 **(12)**	1.378
Hydrocarbons	0.262 **(16)**	0.006 **(10)**	–	0.025 (**10**)	0.331 **(6)**	0.624
Sterols	0.396 **(7)**	0.0074 **(2)**	–	0.019 **(2)**	0.038 **(2)**	0.460
Alkaloids	–	–	–	0.061 **(4)**	0.338 **(4)**	0.399
Others	0.11 **(2)**	0.002 **(1)**	0.019 (1)	0.132 **(6)**	0.780 **(15)**	1.043
Sum	1.8	0.17	1.056	0.75	4.826	8.58
Total no. of identified compounds	50	28	12	52	56	

The bold characters next to each value are the number of compounds detected in each chemical group with different extraction solvents.

from 10.01, 57.57, 2.17, 11.45 to 4.88% of the total peak area depending on the extracting solvent. The total terpene compounds represented about 0.629% DW (Table 1), represented by β-Ionon-5,6-epoxide, dihydroactinidiolide, pristane, (–)–loliolide, Neophytadiene, phytane and phytol (Table 2). Neophytadiene and phytol were the dominant terpenes and represented 149.8 and 208.17 mg/100 g dry biomass. Neophytadiene and phytol were detected in several plants and some microalgae (Mudge and Norris, 1997; Jeng and Huh, 2004; Venkata et al., 2012). Neophytadiene was identified as strong bactericidal, antifungal, antipyretic, analgesic, antioxidant and vermifugic (Venkata et al., 2012), while, phytol that is used in the fragrance industry, cosmetics, shampoos, toilet soaps, household cleaners, and detergents showed antimicrobial, anticancer, antidiuretic activity and was found to be a precursor of vitamin E and vitamin K (Daines et al., 2003 and Netscher, 2007; McGinty et al., 2010). The ionone derivatives, dihydroactinidiolide and pristine were detected in some marine and freshwater algae (e.g. *Spirogyra* species) (Sakagami et al., 1991; Rzama et al., 1995; Kamenarska et al., 2000) and these results are in agreement with the terpene compounds detected in the investigated *S. longata* biomass. The antibacterial effect of many furanone derivatives (e.g. dihydroactinidiolide) was well established (Lorimer et al., 1995; Mendling and Mailland, 2002; Ravikumar et al., 2005). Meanwhile, loliolide was detected in many red and brown marine algae, but it is not a common compound in the green algae. It was its first time to be detected in green algae in the marine green alga *Enteromorpha compressa* (Percot et al., 2009). This monoterpene, loliolide, represents about 112.8 mg/ 100 g dry biomass of *S. longata*. Loliolide is an effective antioxidant against the free radicals 2,2-diphenylpicrylhydrazyl (DPPH), H_2O_2 and intercellular reactive oxygen species (ROS) (Yang et al., 2011).

Phenolic compounds

GC–MS analysis of *S. longata* crude extracts revealed the presence of phenolic compounds in petroleum ether, methylene chloride and chloroform extracts, with a total concentration of 0.654% DW (Table 1), while they were absent in acetone and methanol extracts (Table 2). The variation in the percentage of total phenolic compounds with the extraction solvent, from 55.23% in chloroform extract to 0.37% in petroleum ether (Table 2), reflects the main role of solvent polarity in the extracting process. This is in agreement with the observations of Dent et al. (2013) who reported that, the recovery of phenolic compounds was dependent on the type of solvent used, its polarity and the solubility of phenolic compounds in the extraction solvents. In addition, solvent polarity plays a key role in increasing phenolic solubility (Naczk and Shahidi, 2006). GC–MS analysis revealed the presence of butylated hydroxytoluene (BHT) as a dominant compound in chloroform extract, and represents about 580.6 mg/100 g biomass. It has been reported that this antioxidant, BHT, has many important uses like its usage as a food additive, in which it could prevent lipid oxidation (Wanita and Lorenz, 1996). Also, it could decrease the risk of developing chronic diseases (Gale et al., 1995; Hoffman and Garewal, 1995). BHT was reported to have been incorporated in the industry of diverse products as cosmetics, pharmaceuticals, rubber, electrical transformer oil and embalming fluid (Dugan, 1963; Lauffer, 1972).

Generally, polyphenolic compounds are among the most interesting antioxidant compounds isolated from marine resources, including micro- and macroalgae. At least 8000 different bioactive compounds are considered to be polyphenols (Bravo, 1998). Phenolic compounds were reported to have many pharmacological properties (e.g. anticarcinogenic and antimicrobial activities and effects against neurodegenerative pathologies) (Kono et al., 1995; Esposito et al., 2002; Oueslatia et al., 2012). Junthip et al. (2013) reported that, the antioxidant capacity of a *Spirogyra* species aqueous and methanolic extract is directly related to their phenolic content. In addition, Jung et al. (2003) and Li et al. (2009) contributed the antioxidant action of phenolic compounds, to their high tendency to chelate metals (e.g. iron and copper) by their hydroxyl and carboxyl groups.

Fatty acids (FA)

Fatty acids serve as energetic substrates and allelophatic agents; their antibacterial effect is also well known (McGaw et al., 2002). Saturated fatty acids are synthesized by both plants and animals from acetyl coenzyme A as a form of

Table 2 Ketones, terpenes and phenolic compounds detected in different extracts of *Spirogyra longata*.

Compound (IUPAC-Name)	Common name	Molecular Formula	Retention time (Min.)	% Similarity	Molecular ion peak (m/z)	Peak area%				
						Petroleum ether	Methylene chloride	Chloroform	Acetone	Methanol
Ketones										
3-Penten-2-one, 4-methyl-		$C_6H_{10}O$	3.32	91	98	–	–	–	3.68	17.02
2-Pentanone, 4-hydroxy-4-methyl-		$C_6H_{12}O_2$	3.85	78	116	–	–	–	17.16	6.39
2-Heptanone, 4-methyl-		$C_8H_{16}O$	8.47	80	128	–	–	–	0.77	0.92
3,5,5-Trimethyl-2-cyclohexene-1-one	Isophorone	$C_9H_{14}O$	10.48	79	138	–	–	–	0.48	0.56
2,4,4-trimethyl-3-(3-oxo-1-butenyl)-2-Cyclohexen-1-one		$C_{13}H_{18}O_2$	17.72	98	207	–	–	–	0.182	–
2-Pentadecanone, 6,10,14-trimethyl-	Hexahydrofarnesyl acetone	$C_{18}H_{36}O$	19.92	99	268	10.84	1.76	17.38	6.64	2.5
	Sum					10.84	1.76	17.38	28.91	10.84
Terpenes										
3-Buten-2-one, 4-(2,2,6-trimethyl-7-oxabicyclo [4.1.0] hept-1-yl)-	β-Ionon-5,6-epoxide	$C_{13}H_{20}O_2$	14.97	95	208	0.21	–	–	0.34	–
(7a*R*)-5,6,7,7a-Tetrahydro-4,4,7a-trimethyl-2(4*H*)-benzofuranone	Dihydro-actinidiolide	$C_{11}H_{16}O_2$	15.75	96	180	3.17	0.47	–	1.88	0.54
2,6,10,14-Tetramethylpentadecane	Pristane	$C_{19}H_{40}$	18.38	84	268	–	–	–	0.29	–
(–)–Loliolide		$C_{11}H_{16}O_3$	19.23	95	196	–	2.35	0.83	2.58	1.66
Neophytadiene		$C_{20}H_{38}$	19.83	99	278	0.71	24.32	–	3.15	1.48
2,6,10,14-Tetramethylhexadecane	Phytane	$C_{20}H_{42}$	20.81	92	282	–	–	1.34	0.38	–
(2*E*,7*R*,11*R*)-3,7,11,15-tetramethyl-2-hexadecen-1-ol	Phytol	$C_{20}H_{40}O$	23.19	95	296	5.24	30.43	–	2.55	0.84
4,8,12,16-Tetramethyl-heptadecan-4-olide		$C_{21}H_{40}O_2$	25.83	93	324	0.68	–	–	0.28	0.36
	Sum					10.01	57.57	2.17	11.45	4.88
Phenolics										
4-Ethenyl-2-methoxyphenol		$C_9H_{10}O_2$	12.25	95	150	–	1.29	2.17	–	–
Phenol, 3-(1,1-dimethylethyl)-4-methoxy-		$C_{11}H_{16}O_2$	14.73	76	180	–	–	2.36	–	–
Phenol, 2,6-bis(1,1-dimethylethyl)-4-methyl-	Butylated hydroxytoluene (BHT)	$C_{15}H_{24}O$	15.34	97	220	–	7.5	50.7	–	–
Phenol, 2,2′-methylenebis[6-(1,1-dimethylethyl)-4-methyl-		$C_{23}H_{32}O_2$	26.56	98	340	0.37	7.79	–	–	–
	Sum					0.37	16.58	55.23	–	–

Table 3 Fatty acids, fatty alcohols and esters detected in different extracts of *Spirogyra longata*.

Compound (IUPAC-Name)	Common name	Molecular Formula	Retention time (Min.)	% Similarity	Molecular ion peak (m/z)	Peak area%				
						Petroleum ether	Methylene chloride	Chloroform	Acetone	Methanol
Fatty acids										
Tetradecanoic acid	Myristic acid	$C_{14}H_{28}O_2$	18.93	95	228	–	–	–	0.55	1.18
Pentadecanoic acid	Pentadecylic acid	$C_{15}H_{30}O_2$	20.21	96	242	–	–	–	–	0.29
Hexadecanoic acid	Palmitic acid	$C_{16}H_{32}O_2$	21.56	99	256	4.29	–	–	10.23	18.66
9-Octadecenoic acid, (*E*)	Elaidic acid	$C_{18}H_{34}O_2$	23.52	76	282	1.45	0.64	–	0.72	1.07
	Sum					5.74	0.64	–	11.5	21.2
Fatty alcohols										
2-Dodecen-1-ol	Dodecyl alcohol	$C_{12}H_{25}OH$	16.81	92	186	0.18	–	–	–	
1-Hexadecanol	Cetyl or palmityl alcohol	$C_{16}H_{33}OH$	19.50	89	242	0.22	–	–	–	0.52
9,12,15-Octadecatrien-1-ol, (Z,Z,Z)-	Linolenyl alcohol	$C_{18}H_{31}OH$	22.36	83	264	3.48	3.84	–	–	–
1-Octadecanol	Stearyl alcohol	$C_{18}H_{37}OH$	20.37	97	270	3.33	7.77	–	1.22	0.49
1-Icosanol	Arachidyl alcohol	$C_{20}H_{41}OH$	24.82	76	298	–	–	–	2.05	0.25
	Sum					7.21	11.61	–	3.27	1.26
Esters										
Tetradecanoic acid, 12-methyl-, methyl ester		$C_{16}H_{32}O_2$	18.31	97	256	–	–	–	–	0.46
Tetradecanoic acid, methyl ester	Methyl tetradecanoate	$C_{15}H_{30}O_2$	19.16	95	242	0.13	–	–	0.24	0.32
Pentadecanoic acid, methyl ester		$C_{16}H_{32}O_2$	19.65	97	256	–	–	–	0.22	0.42
7,10,13-Hexadecatrienoic acid, methyl ester		$C_{17}H_{28}O_2$	20.61	97	264	0.97	–	–	–	0.13
9-Hexadecenoic acid, methyl ester, (Z)-		$C_{17}H_{32}O_2$	20.67	98	268	–	–	–	–	0.17
Hexadecanoic acid, methyl ester	Palmitic acid, methyl ester	$C_{17}H_{34}O_2$	20.93	98	270	0.4	0.23	6.7	0.96	8.95
Hexadecanoic acid, ethyl ester	Palmitic acid, ethyl ester	$C_{18}H_{36}O_2$	21.76	99	284	6.88	–	–	–	–
Heptadecanoic acid, methyl ester	Heptadecanoates	$C_{18}H_{36}O_2$	22.13	94	284	–	–	2.08	–	–
Hexadecanoic acid, 2-hydroxy-, methyl ester		$C_{17}H_{34}O_3$	22.44	93	286	0.25	0.61	–	0.72	–
9,12-Octadecadienoic acid, methyl ester	Oleic acid, methyl ester	$C_{19}H_{34}O_2$	22.95	99	294	3.62	0.48	2.15	0.32	0.35
9,12,15-Octadecatrienoic acid, methyl ester	Linoleic acid methyl ester	$C_{19}H_{32}O_2$	23.06	97	292	1.20	0.72	2.18	0.35	0.73
Octadecanoic acid, methyl ester	Stearic acid, methyl ester	$C_{19}H_{38}O_2$	23.29	99	298	–	–	2.59	0.26	0.46
9,12-Octadecadienoic acid (Z,Z)-, ethyl ester	Linoleic acid ethyl ester	$C_{20}H_{36}O_2$	23.72	91	308	0.81	–	–	0.19	0.71
Eicosanoic acid, methyl ester	Arachidic acid, methyl ester	$C_{21}H_{42}O_2$	25.48	95	326	0.23	–	–	–	–
Hexanedioic acid, bis(2-ethylhexyl) ester	Diisooctyl adipate (DEHA)	$C_{22}H_{42}O_4$	26.23	94	370	1.96	0.76	–	–	–
Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl) ethyl ester		$C_{19}H_{38}O_4$	27.38	99	330	–	–	–	1.10	1.14
Docosanoic acid, methyl ester	Behenic acid, methyl ester	$C_{23}H_{46}O_2$	27.50	98	354	–	–	–	5.80	0.54
Decanedioic acid, bis(2-ethylhexyl) ester	Dioctyl sebacate	$C_{26}H_{50}O_4$	30.10	90	426	–	–	–	2.92	–
Tetradecanoic acid, hexadecyl ester	Cetyl myristate	$C_{30}H_{60}O_2$	34.76	85	452	1.76	–	–	–	–
Hexadecanoic acid, hexadecyl ester	Cetyl palmitate	$C_{32}H_{64}O_2$	39.13	95	480	2.48	–	–	–	–
	Sum					20.67	2.8	16.9	13.08	14.38

Table 4 Hydrocarbons, steroids and other compounds detected in different extracts of *Spirogyra longata*.

Compound (IUPAC-Name)	Common name	Molecular formula	Retention time (min)	% Similarity	Molecular ion peak (m/z)	Peak area%				
						Petroleum ether	Methylene chloride	Chloroform	Acetone	Methanol
Hydrocarbons										
Decane		$C_{10}H_{22}$	14.46	86	142	–	0.68	–	0.22	0.85
Tridecane		$C_{13}H_{28}$	14.54	75	184	–	–	–	0.36	4.32
2-Undecene, 2,5-dimethyl-		$C_{13}H_{26}$	16.13	92	182	0.33	–	–	0.25	–
Tetradecene		$C_{14}H_{28}$	17.63	97	196	1.64	–	–	0.26	–
Cyclotetradecane		$C_{14}H_{28}$	17.67	85	196	–	–	–	0.28	–
1-Pentadecene		$C_{15}H_{30}$	17.95	93	210	4.87	0.5	–	0.6	–
Pentadecane		$C_{15}H_{32}$	18.03	96	212	0.18	0.18	–	–	–
Hexadecane		$C_{16}H_{34}$	16.54	98	226	–	–	–	0.24	0.20
Octadecene		$C_{18}H_{36}$	19.06	98	252	0.89	–	–	–	–
Octadecane		$C_{18}H_{38}$	19.29	98	254	0.36	–	–	–	–
1-Nonadecene		$C_{19}H_{38}$	20.48	99	266	1.64	0.26	–	0.45	0.44
Nonadecane		$C_{19}H_{40}$	20.57	97	268	0.97	–	–	–	–
Heneicosane		$C_{21}H_{44}$	22.97	98	296	0.21	–	–	0.41	0.51
Octadecane, 2,6,10,14-tetramethyl-		$C_{22}H_{46}$	24.09	95	310	0.38	–	–	–	0.53
Cyclotetracosane		$C_{24}H_{48}$	24.21	98	336	0.22	0.24	–	–	–
Docosane		$C_{22}H_{46}$	25.16	95	310	0.57	0.21	–	–	–
Pentacosane		$C_{25}H_{52}$	27.19	98	352	0.32	0.25	–	0.26	–
Heptacosane		$C_{27}H_{56}$	28.13	97	380	0.39	0.34	–	–	–
Octacosane		$C_{28}H_{58}$	29.06	98	394	0.57	0.39	–	–	–
Nonacosane		$C_{29}H_{60}$	30.99	99	408	0.72	0.27	–	–	–
	Sum					14.26	3.32	–	3.33	6.85
Sterols										
Cholestan-3-ol, (3β,5β)-	Coprostanol	$C_{27}H_{48}O$	33.60	90	388	1.43	–	–	1.33	0.38
Cholest-5-en-3-ol, (3β)-		$C_{27}H_{46}O$	34.24	99	386	1.99	–	–	–	–
Cholestanol		$C_{27}H_{48}O$	34.39	97	388	0.49	–	–	–	–
Ergost-5-en-3-ol, (3β,24R)-	Campesterol	$C_{28}H_{48}O$	36.18	99	400	0.59	4.0	–	–	–
Stigmasta-5,22-dien-3-β-ol		$C_{29}H_{48}O$	36.84	99	412	1.37	–	–	–	–
γ-Sitosterol		$C_{29}H_{50}O$	38.18	99	414	15.04	0.32	–	1.21	0.35
Stigmastan-3,5-diene		$C_{29}H_{48}$	39.72	98	396	0.62	–	–	–	–
	Sum					21.53	4.32	–	2.54	0.73
Alkaloids										
2-Methylpyridine	α-Picoline	C_6H_7N	3.58	93	93	–	–	–	0.35	–
2,4-dimethylpyridine	2,6-Lutidine	C_7H_9N	5.24	87	107	–	–	–	–	0.82
4-Piperidinone, 2,2,6,6-tetramethyl-		$C_9H_{17}NO$	8.59	90	155	–	–	–	4.97	3.44
8-methylquinoline		$C_{10}H_9N$	9.59	58	143	–	–	–	0.89	1.41

(*continued on next page*)

Table 4 (*continued*)

Compound (IUPAC-Name)	Common name	Molecular formula	Retention time (min)	% Similarity	Molecular ion peak (m/z)	Peak area%				
						Petroleum ether	Methylene chloride	Chloroform	Acetone	Methanol
1H-Pyrrole-2,5-dione, 3-ethyl-4-methyl-		$C_7H_9NO_2$	10.86	91	139	–	–	–	1.94	1.32
	Sum					–	–	–	8.15	6.99
Others										
Unknown 1			6.23	43	117	–	–	–	6.75	2.33
Unknown 2			8.98	34		–	–	–	–	0.45
Unknown 3			9.26	22		–	–	–	–	1.59
Unknown 4			10.06	45		–	–	–	–	0.60
Unknown 5			10.15	42		–	–	–	–	0.42
Unknown 6			12.7	33		–	–	–	0.33	0.87
Unknown 7			13.09	45		–	–	–	–	0.61
Unknown 8			13.34	22		–	–	–	–	0.69
Unknown 9			14.00	34	168	–	–	–	0.33	1.68
Unknown 10			15.02	40		–	–	1.3	–	–
Tetradecanal	Myristyl aldehyde	$C_{14}H_{28}O$	18.21	91	212	1.41	–	–	–	–
Unknown 11			19.5	13		–	–	–	–	0.48
Unknown 12			23.84	16		–	–	–	–	0.53
(Z)-Octa-9-decenamide	Oleylamide	$C_{18}H_{35}NO$	24.02	60	281	–	–	–	0.24	0.20
Unknown 13			24.33	35		3.54	0.89	–	–	–
Unknown 14			27.08	16		–	–	–	–	0.63
Unknown 15			27.39	32		–	–	–	0.92	0.85
Bis(2-ethylhexyl) phthalate	EHP	$C_{24}H_{38}O_4$	27.74	90	390	–	–	–	8.00	2.95
	Sum					4.95	0.89	1.3	16.57	14.88

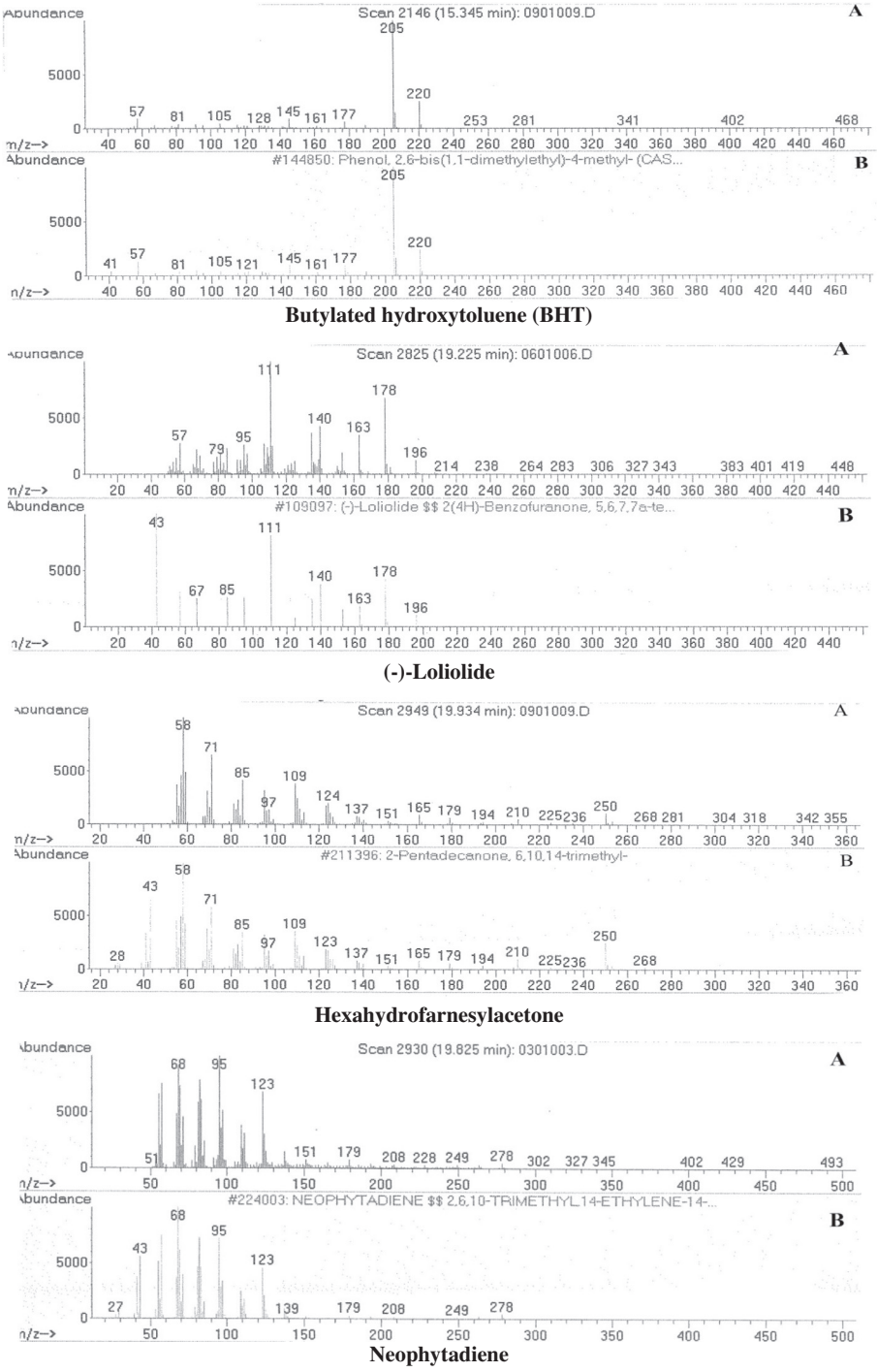

Figure 3a Mass spectra of butylated hydroxytoluene (BHT), (-)-loliolide, hexahydrofarnesylacetone and neophytadiene detected in different extracts of *Spirogyra longata*. A = compound in the extracts, B = compound in the library.

long-term energy storage. The saturated fatty acids have been found to affect the hypercholesterolemia and induce the expression of cyclooxygenase-2 (Lee et al., 2001). The concentration of fatty acids in the investigated alga represents about 1.22% DW (Table 1), detected in petroleum ether, methylene chloride, acetone and methanol extracts with total peak area% of 5.74, 0.64, 11.5 and 20.2 (Table 3). As shown in Table 3, the fatty acid composition of *S. longata* was represented by three saturated fatty acids; tetradecanoic acid, pentadecanoic acid, hexadecanoic acid and one monounsaturated omega-9 (n-9) fatty acid (elaidic acid; $C_{18}H_{34}O_2$) (Table 3). Elaidic acid is the trans isomer of oleic acid which is found in hydrogenated vegetable oils. It also occurs in small amounts in caprine and bovine milk (very roughly 0.1% of the fatty acids) (Alonso et al., 1999). This type of fatty acid was reported to increase the plasma cholesteryl ester transfer protein (CETP) activity which lowers high-density lipoproteins (HDL) cholesterol (Abbey and Nestel, 1994). Palmitic acid and elaidic acid were

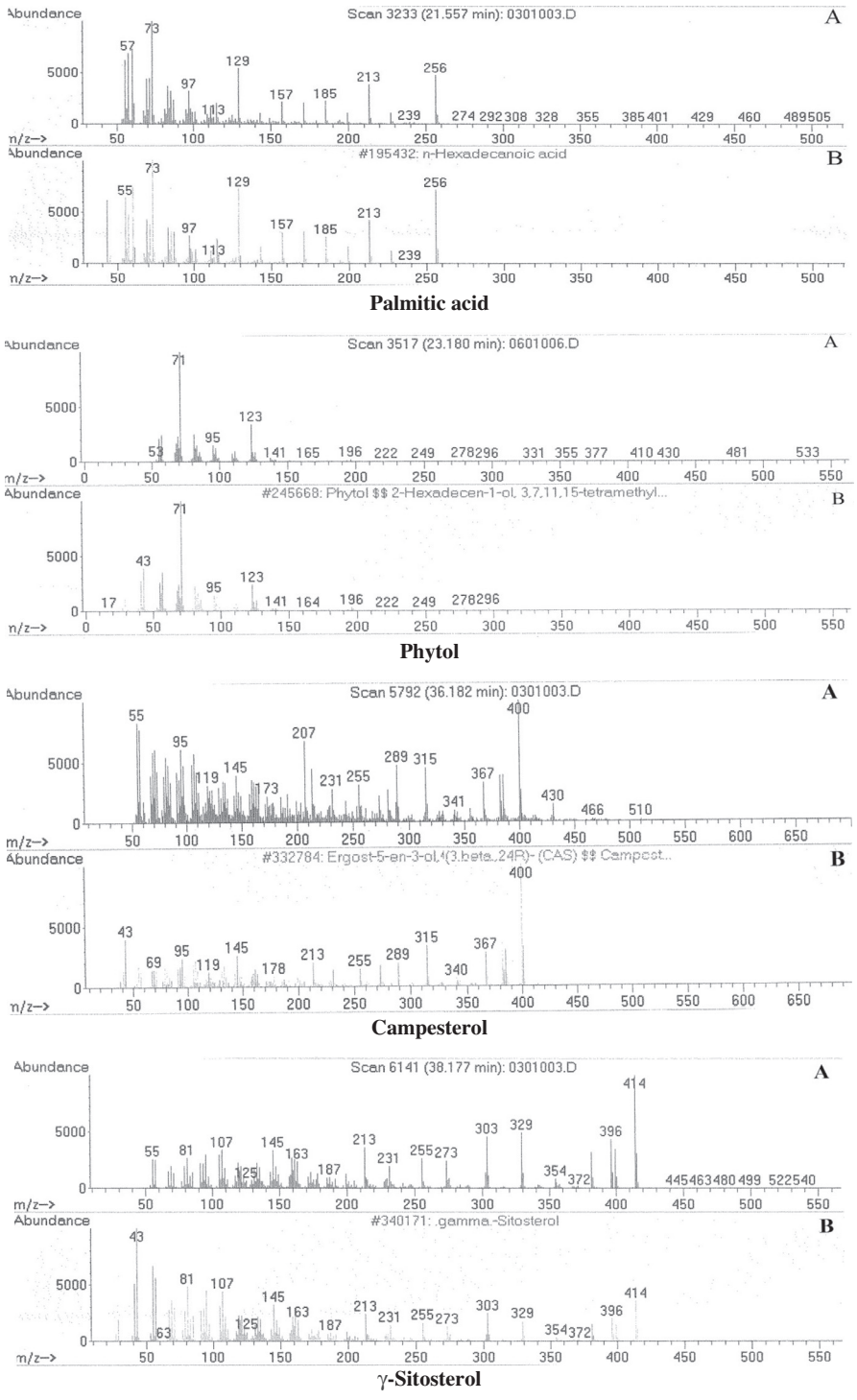

Figure 3b Mass spectra of palmitic acid, campesterol, phytol and γ-sitosterol detected in different extracts of *Spirogyra longata*. A = compound in the extracts, B = compound in the library.

the dominant FA in *S. longata* extracts. The fatty acid profile in the investigated alga was in agreement with that reported by Ivanova et al. (2002) in the same *Spirogyra* species.

Fatty alcohols

Fatty alcohols are naturally derived from plant or animal lipids and used in pharmaceutical, detergent or plastic industries (Noweck and Grafahrend, 2006). Fatty alcohols in *S. longata* were represented by four saturated fatty alcohols; dodecyl alcohol, palmityl alcohol, stearyl alcohol, arachidyl alcohol and one polyunsaturated fatty alcohol (linolenyl alcohol) (Table 3). Linolenyl alcohol and stearyl alcohol were the dominant fatty alcohols in petroleum ether and methylene chloride extracts (Table 3). The saturated fatty alcohols are chemical intermediates for surfactants and have a wide range

of uses as ingredient in personal care and home care products, pharmaceutical formulations, agrochemicals and industrial uses (Noweck and Grafahrend, 2006).

Esters

Twenty different ester compounds were identified in the different crude extracts of *S. longata*. Out of them, 19 compounds were derived from fatty acids and only one ester was derived from the dicarboxylic acid, decanedioic acid (Table 3). The decanedioic acid, bis(2-ethylhexyl) ester was identified only on the acetone extract with a peak area% of 2.92 (Table 3). The total number of ester compounds represented about 1.38% DW (Table 1). Mainly, esters derived from palmitic acid were the dominant esters in different crude extracts of *S. longata* and represented about 36.92%, 8.21%, 49.6%, 7.34 and 62.24% of the total ester peak area percentage of petroleum ether, methylene chloride, chloroform, acetone and methanol extracts (Table 3). Some of other esters detected in different extracts of *S. longata* found to contain useful applications, for example, ethyl oleate and ethyl stearate. Ethyl oleate is used as a solvent for pharmaceutical drug preparations (Ory et al., 1983). It was also reported as a primer pheromone in honeybees (Leoncini et al., 2004). Meanwhile, stearic acid ethyl ester was reported to perturb the cell cycle and induces apoptosis in Hep-G2 cells and is a marker of excessive alcohol consumption that can be isolated from an individual's hair (Aydin et al., 2005). Kamenarska et al. (2000) reported the absence of methyl eaters in the methanolic extracts from the same investigated species of *Spirogyra*, however, he reported that the formation of methyl esters proved to be artifacts, in which, they formed through the extraction procedure by methanolysis of the lipids. In this context, the different extracts of *S. longata* were found to contain a number of polyunsaturated fatty acids (PUFA). They were represented as 7,10,13-hexadecatrienoic acid (HTA, n-3), 9-hexadecenoic acid (n-7), 9,12-octadecadienoic acid (n-6), 9,12,15-octadecatrienoic acid (n-3) and 9,12-octadecadienoic acid (Z,Z)-(n-3) (Table 3). PUFAs were reported to play key roles in cellular and tissue metabolism, including the regulation of membrane fluidity, electron and oxygen transport, thermal adaptation and could reduce coronary heart disease risk (Funk, 2001; Mozaffarian et al., 2005). Also, the presence of methyl palmiteate (C16:0), methyl palmitoleate (C16:1) and methyl linoleate myristate (C18:2) in extracts of *S. longata* indicated their suitability for biodiesel production (Chisti, 2007; Hu et al., 2008).

Hydrocarbons

The hydrocarbon content in *S. longata* varied greatly between different extraction solvents, in which it represented a total peak area% of 14.26, 3.32, 3.33 and 6.85 for petroleum ether, methylene chloride, acetone and methanol extracts (Table 4). The identified hydrocarbons ranged from C_{10} to C_{29}, and were dominated by the odd number carbon atoms (C_{13}, C_{15}, C_{19}, C_{21}, C_{25}, C_{27} and C_{29}). Most of the identified hydrocarbons were alkanes, in which, out of the identified 20 hydrocarbons, 15 compounds were alkanes (Table 4). The hydrocarbon composition in the investigated *S. longata* was in agreement with that reported by Kamenarska et al. (2000) in the same investigated *Spirogyra* species. The hydrocarbon compounds detected in *S. longata* represented about 0.624 g/100 g dry alga biomass (Table 1). Hydrocarbons of microalgae represented a potential feedstock of hydrocarbon fuels. It is suggested that hydrocarbons from microalgae have contributed to oil deposits throughout the world (Traverse, 1955; Moldowan and Seifert, 1980). In addition, some hydrocarbons may play a role in the chemical communication of several insects, for example, nonacosane is known to be a pheromone of female *Anopheles stephensi* mosquito (Brei et al., 2004). Nonacosane was detected in the investigated *S. longata* petroleum ether and methylene chloride extracts with a peak area% of 0.72 and 0.27 (Table 4).

Sterols

Sterols and some of their derivatives were previously found to have played an important role in lowering LDL cholesterol levels in vivo (Francavilla et al., 2010). In addition, phytosterols (C_{28} and C_{29} sterols) are important precursors of compounds (e.g. D vitamins) (Kametani and Furuyama, 1987). Phytosterols in *S. longata* were represented by 7 different steroid compounds, that were mainly concentrated in the petroleum ether extract (Table 4). Sterols maintained about 0.46% DW of *S. longata* (Table 1), and represented by a total peak area% of 21.53, 4.32, 2.54 and 0.73 of petroleum ether, methylene chloride, acetone and methanol extracts (Table 4). Mitova et al. (1999) reported the presence of a variety of sterols in several species of *Spirogyra*. The profile of sterols in the investigated alga was in agreement with that reported by Omer (2013) in the same *Spirogyra* species. *S. longata* found to contain a variety of stigmasterol compounds (Table 4). Stigmasterol is one of the phytosterols that include β-sitosterol, campesterol, ergosterol (precursor of vitamin D2 and cortisone), brassicasterol, delta-7-stigmasterol and delta-7-avenasterol (Kanimozhi and Ratha, 2012). Research on stigmasterol indicated that it possesses potent antioxidant, hypoglycemic and thyroid inhibiting properties (Panda et al., 2009). Stigmasterol compounds were also reported to have valuable roles in the regulatory and tissue rebuilding mechanisms related to estrogen effects, because they act as a precursor in the manufacture of the human hormone (progesterone). Other important roles of stigmasterol are acting as an intermediate in the biosynthesis of androgens, estrogens, and corticoids (Sundararaman and Djerassi, 1997).

Alkaloids and others

Twenty-three compounds belonging to different chemical groups were identified in the extracts of *S. longata* and are shown in Table 4, namely 5 alkaloids, 1 amide (Octa-9-decenamide; amide of oleic acid), 1 aldehyde (Tetradecanal), 1 phthalate derivative (Bis(2-ethylhexyl) phthalate) and 15 unknown compounds. Alkaloids present special interest because of their pharmacological activities. Many reports revealed the presence of alkaloids in marine algae and some of them were investigated for their biological activity (Guven et al., 2010). Yousif et al. (2014) recorded the presence of terpenoid, flavonoids, phenols saponins and alkaloids in the alcoholic extract of *Spirogyra* species. In the present study, alkaloids were detected only in acetone and methanol extracts (Table 4). In addition, Omer (2013) reported the presence of five types of alkaloid compounds in *Spirogyra*. However, the

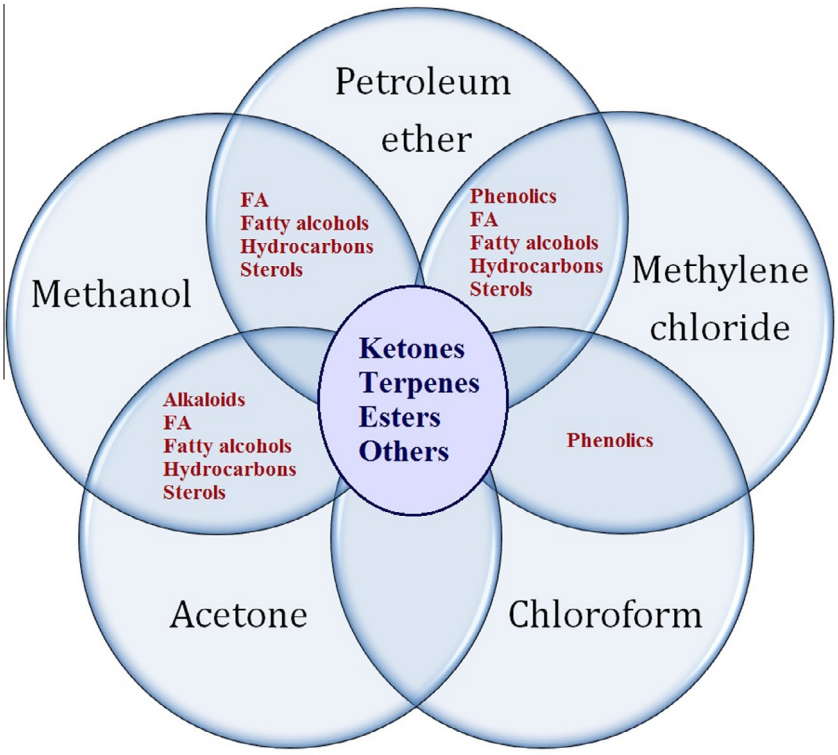

Figure 4 Venn diagram elucidate the overlapping of compounds belonging to different identified chemical groups between different extraction solvents.

detected alkaloids in the investigated alga *S. longata* did not match the results of Omer (2013).

The correlation between the extraction solvent and the extracted molecules is elucidated by the Venn diagram shown in Fig. 4. Obvious correlation was detected between the extraction solvent and the identified chemical groups especially for phenolics and alkaloids, in which phenolics overlapped between petroleum ether, methylene chloride and chloroform. Meanwhile, alkaloids overlapped between acetone and methanol, indicating that the extraction of compounds belonging to this chemical group requires solvent with polarity higher than 5.1 (polarity of acetone and methanol). The other interesting observation is that, hydrocarbons, FA, fatty alcohols and sterols were not extracted by chloroform (Fig. 4). However, they were extracted by acetone and methanol solvents, which had polarity higher than chloroform. This may indicate that the extracted molecules were affected not only by the solvent's polarity but also by the other properties of the extraction solvent (not studied in the present work).

Conclusion

The present results indicated the presence of many valuable compounds like, alkaloids, ketones, terpenes, phenolics, hydrocarbons, fatty acids, fatty alcohols and esters in the different *S. longata* extracts. The extraction solvent found to affect the extracted molecules, especially alkaloids phenolics and sterols. The detection of many antioxidants and PUFA (n-3, n-6, n-7 and n-9) in *S. longata* revealed that it could be used as a source of nutraceuticals in food and feed for humans and animals. Also, the high content of hydrocarbons and fatty acids revealed the potential of *S. longata* lipids when used as a feedstock of biodiesel and hydrocarbon fuels. In addition, the worldwide distribution of *S. longata* presents an opportunity to obtain theses valuable compounds, but the sustainable production of these compounds on a large scale may be tuned by further research of selecting appropriate cultivation conditions for this valuable algal species.

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